

Chromogranin A as a marker of neuroendocrine neoplasia: an Italian Multicenter Study

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Abstract

Elevated circulating chromogranin A (CgA) levels are found in neuroendocrine tumors (NETs), but the diagnostic usefulness of this marker is still debatable. To assess the role of CgA for the diagnosis of gastroenteropancreatic (GEP) NETs and the identification of metastatic patients, an Italian multicenter observational study has been performed. CgA was evaluated in 202 GEP NET patients by IRMA and ELISA. The cutoffs for diagnosis and presence of metastases were identified by receiver-operating characteristic (ROC) curve. We found good correlation between IRMA and ELISA. The ROC analysis identified a cutoff of 53 ng/ml for IRMA and 16 U/l for ELISA as discriminating between controls and patients with active disease (sensitivity 71.3 and 84%; specificity 71 and 85% respectively). Metastases were present in 123 patients, having significantly higher CgA levels than patients without metastases. ROC analysis identified a cutoff of 146 ng/ml for IRMA and 67.3 U/l for ELISA as discriminating between patients with and without metastases (sensitivity 57 and 63.3%; specificity 55.6 and 71.4% respectively). For pancreatic NETs positive and negative predictive values were 84 and 78% respectively (90% specificity and 68% sensitivity). We found lower CgA levels in patients with extensive metastatic spread than in those with liver metastases only. These data assess the role of CgA evaluation in GEP NETs, and demonstrate that higher CgA levels associate with metastatic disease, confirming that CgA levels can provide a helpful practical biochemical marker for the clinical management of NETs, but with low sensitivity and specificity.

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Introduction

Chromogranin A (CgA) is an acidic glycoprotein expressed in the secretory granules of most normal and neoplastic neuroendocrine (NE) cell types, where it is released together with peptide hormones and biogenic amines (Taupenot *et al.* 2003). Elevated circulating CgA levels have been demonstrated in serum or plasma of patients with various NE tumors (NETs; Nobels *et al.* 1997, Guignat *et al.* 2001, Tomassetti *et al.* 2001).

Previous studies reported different ranges of sensitivity and specificity for circulating CgA, according to histological characteristics of the tumor and to disease spread. These parameters have been demonstrated to depend also on the method used for serum or plasma CgA determination and on the threshold considered as pathologic (Schürmann *et al.* 1992, Stridsberg *et al.* 1995, Nobels *et al.* 1997, Baudin *et al.* 2001, Stivanello *et al.* 2001, Tomassetti *et al.* 2001). In order to clarify this

issue, an Italian multicenter observational study has been performed in a large series of gastroenteropancreatic (GEP) NET patients and healthy controls to assess the usefulness of CgA determination for the diagnosis of sporadic GEP NETs and to establish the best cutoff value for the diagnosis of GEP NETs using the method of the receiver-operating characteristic (ROC) curve.

Materials and methods

Subjects

The control group was composed of 129 healthy individuals (61 males and 68 females; aged 44.2 ± 8.4 years (mean \pm S.D.); range 22–59 years) without evidence of NETs, malignancies, hypertension, renal or liver failure, and not treated with proton pump inhibitors.

Overall, 273 patients with NETs were enrolled between April 2003 and October 2004 in 40 different Italian centers, participating to the CROMaNET study, a multicenter observational study for the evaluation of CgA as marker for diagnosis and follow-up of NET. Among these subjects, 202 patients (109 males and 93 females; aged 58.5 ± 13.8 years; range 14–84 years) were diagnosed from 1 to 120 months earlier (median 45 months) with GEP NET, pathologically proven by histological and immunohistochemical diagnosis after surgery or biopsy of primary tumor or metastases.

Exclusion criteria were kidney defect (plasma creatinine $> 120 \mu\text{M/l}$; Canale & Bravo 1994), liver failure, treatment with proton pump inhibitors, Parkinson's disease, pregnancy, or the presence of any other malignancy.

The GEP NET group included 73 patients with primitive NET site in the pancreas, 2 in the esophagus, 27 in the stomach, 7 in the duodenum, 71 in the ileum, 14 in the colon, and 8 in the rectum. Conventional imaging (abdominal and thoracic CT and/or MRI), as well as ultrasonography, endoscopy, echo-endoscopy, and somatostatin receptor scintigraphy (Octreoscan) were used for staging when appropriate. Among these patients, 123 (60.9%) presented with metastases. The extent of metastatic spread was defined as locally advanced (when limited to regional lymph nodes), with liver metastases (when only liver metastases were evident) and with liver and extra-hepatic metastases (when bone, lung, or brain metastases were demonstrated). Patients were divided into four groups:

- 1) new diagnosis (ND, 81 patients): including patients diagnosed at the centre for the first time as having a GEP NET, with evidence of disease at study entry;

- 2) relapse (RL, 27 patients): patients with evidence of recurrent disease, not medically treated for at least 6 months before study entry;
- 3) stable disease (SD, 49 patients): patients with evidence of persistent disease, medically treated for at least 6 months before study entry;
- 4) remission (RM, 45 patients): patients previously treated (either surgically or medically) for a GEP NET, with no evidence of disease at study entry.

CgA determination

All samples were collected after an overnight fast, as previously described (Leon et al. 2005), for plasma and serum, both aliquoted and stored at -80°C . Measurement of serum CgA levels was performed between February and July 2005, both locally and in two reference laboratories, by a two-step IRMA (IRMA; CGA-RIA CT, CIS-bio international-Shering, Gif-sur-Yvette, France) in Venezia (ABO Association c/o Regional Center for the Study of Biological Markers of Malignancy, General Regional Hospital, Venezia, Italy) and by ELISA (DAKO Cytomation, Glostrup, Denmark) in Orbassano (Medical Oncology Unit, S. Luigi Hospital, Orbassano, Torino, Italy). Both methods were performed according to the manufacturer's instructions. All samples were assayed in duplicate by the same technician.

The IRMA assay is based on two monoclonal antibodies raised against the unprocessed central domain (CgA_{145–245}) of the human CgA, allowing sensitive detection of total human CgA. Recombinant human CgA was used as calibrator and the standard curve concentrations ranged from 22 to 1200 ng/ml, with a minimal detectable level of 10 ng/ml. Inter-assay coefficients of variation were 3.4 and 4.5% at 124.7 and 355.2 ng/ml respectively. Intra-assay coefficients of variation were 5.1, 3.0, and 7.8% for the following ranges 15–25, 90–110, and 500–700 ng/ml respectively.

The ELISA assay is based on two polyclonal rabbit antibodies directed towards a 23 kDa carboxyl-terminal fragment of human CgA, therefore measuring more human CgA fragments. The calibrators were extracted from urine of patients with carcinoids and the standard curve concentrations ranged from 5 to 650 U/l, with a minimal detectable level of 5 U/l. Inter-assay coefficients of variation were 3.4, 3.9, and 6.8% at 11.5, 52.7, and 358 U/l respectively. Intra-assay coefficients of variation were 4.5, 3.8, and 8.5% for the following ranges 5–10, 15–25, and 250–450 U/l respectively.

Statistical analysis

CgA levels are reported as the mean \pm s.d., the median, and the range for both IRMA and ELISA methods. Comparisons of values from independent groups were performed using the nonparametric test of Wilcoxon. To measure the strength of association between pairs of variables without specifying dependencies, Spearman order correlations were run. A $P < 0.05$ was considered significant in all tests.

In order to identify a cutoff CgA value for both IRMA and ELISA assays that could discriminate between controls and patients, a ROC curve was constructed using CgA levels from the 129 controls and those from 81 ND patients with GEP NETs, which were considered as having the disease at the moment of blood sampling. In order to identify a cutoff CgA value for both IRMA and ELISA assays that could discriminate between patients without and with metastases, a ROC curve was constructed using CgA levels from 29 patients without metastases and those from 79 metastatic patients belonging to the ND and RL groups (108 patients), all considered as having the disease at the moment of blood sampling.

ROC analysis was performed using a statistical software package (SAS, version 8.2). The area under the ROC curve (AUC) was calculated to describe the capability of the marker to discriminate between patients and controls. Sensitivity and specificity were calculated for different cutoff values. The optimal value giving the best compromise between sensitivity and specificity was chosen to analyze the performance of CgA assays in GEP NET patients. Sensitivity and specificity were calculated using the standard formulae (sensitivity % = true positive / true positive + false negative and specificity % = true negative / true negative + false positive). The correction for the disease prevalence was adopted to calculate positive predictive value (PPV) and negative predictive value (NPV): PPV (%; probability that a positive value corresponds to a true positive result) = sensitivity / sensitivity + (1 – specificity) / prevalence of disease and NPV (%; probability that a negative value corresponds to true negative result) = specificity / specificity + (1 – sensitivity) \times prevalence of disease.

Results

CgA levels in healthy subjects and in GEP NET patients

CgA levels, assessed by both IRMA and ELISA methods, were highly variable and not normally distributed among the 129 healthy subjects (Table 1)

Table 1 Basal chromogranin A levels in 129 healthy subjects assessed by IRMA and ELISA

	IRMA (ng/ml)	ELISA (U/l)
No.	129	129
Mean	46.9	12.3
s.d.	31.2	10.1
Median	40.0	10.4
Range	17–269	5–106.5
Percentile		
5th	21.0	6.2
25th	31.0	8.0
50th	40.0	10.4
75th	54.0	13.7
95th	86.0	19.3

and in the 202 GEP NET patients (Table 2). The analysis of collected data showed a good correlation between IRMA and ELISA assays in measuring CgA levels both in healthy subjects ($r = 0.689$; $P < 0.0001$; Fig. 1A) and in GEP NET patients ($r = 0.848$; $P < 0.0001$; Fig. 1B). In addition, a good correlation between local and central laboratories in measuring CgA levels both with IRMA ($r = 0.846$; $P < 0.0001$; Fig. 2A) and with ELISA assays ($r = 0.873$; $P < 0.0001$) was found (Fig. 2B).

Diagnostic property of CgA

In order to identify a cutoff value that could distinguish between healthy subjects and affected patients, we performed a ROC analysis considering CgA levels from the 129 controls and those from 81 ND patients with GEP NETs, measured by both IRMA and ELISA assays.

As shown in Fig. 3A, the cutoff value of 53 ng/ml for the IRMA assay provided the best compromise between specificity (71.3%) and sensitivity (77.8%), and was chosen for further analysis. The area under the curve (AUC) was 0.834, indicating a good performance of the assay. Using this cutoff, PPV were 54 and 35% and NPV were 92 and 90% for foregut (esophagus, stomach, pancreas, and duodenum) and midgut tumors (ileum and colon) respectively. Analysis of these parameters was then performed according to the affected organ. Due to the low number of the GEP NET patients with primary site of the tumor in the duodenum, colon, and rectum, PPV and NPV were only calculated for stomach (27 and 99%), pancreas (43 and 93%), and ileum (29 and 92%) respectively. CgA levels were below the cutoff value in 96 out of 129 normal individuals (74.4%) and in 28 out of 45 patients (62%) with endocrine tumors in RM.

Table 2 Basal chromogranin A levels in 202 gastroenteropancreatic neuroendocrine tumor patients according to the recruitment group

	New diagnosis		Relapse		Stable disease		Remission	
	IRMA (ng/ml)	ELISA (U/l)	IRMA (ng/ml)	ELISA (U/l)	IRMA (ng/ml)	ELISA (U/l)	IRMA (ng/ml)	ELISA (U/l)
No.	81	81	27	27	49	49	45	45
Mean	546.1	349.2	624.6	364.4	434.6	160.5	67.3	19.0
S.D.	1496.5	1020.6	872.8	639.7	1441.7	375.5	82.0	14.1
Median	164	67	216	104	57	30	43	15
Range	10–11 720	5–8170	36–3045	7–3018	10–9555	5–2489	10–543	5–66
Percentile								
5th	28	9	39	8	19	6	23	6
25th	66	27	69	41	34	15	29	11
50th	164	67	216	104	57	30	43	15
75th	342	215	971	476	132	171	76	24
95th	1410	1423	3040	1551	1745	562	159	53

As shown in Fig. 3B, the cutoff value of 16 U/l for the ELISA assay provided the best compromise between specificity (85.3%) and sensitivity (84%), and was chosen for further analysis. The AUC was 0.892, again indicating a good performance of the assay. Using this cutoff, PPV were 69 and 57% and NPV were 93 and 96% for foregut and midgut tumors respectively. When considering the primary site of the tumor, PPV and NPV were 42 and 99% for stomach, 60 and 94% for pancreas, and 51 and 97% for ileum respectively. CgA levels were below this cutoff value in 110 out of 129 normal individuals (85.3%) and in 25 out of 45 patients (55.6%) with endocrine tumors in RM.

Considering these cutoff levels, six healthy subjects having normal CgA levels by IRMA had CgA levels above the cutoff when assayed by ELISA. On the contrary, 20 healthy subjects having normal CgA by ELISA had CgA levels above the cutoff when assayed by IRMA (discordance rate 20.1%).

CgA levels in metastatic patients

Among the 202 GEP NET patients, 123 presented with and 76 without metastases at study entry. Data concerning the presence or absence of metastases were missing in three patients. Metastatic patients had significantly ($P<0.0001$) higher CgA levels than patients without metastases, both by IRMA (605.9 ± 1537.9 ng/ml, range 10–11, 720 ng/ml vs 142.1 ± 324.6 ng/ml, range 10–2715 ng/ml) and by ELISA assays (351.5 ± 899.3 U/l, range 5–8170 U/l vs 47.7 ± 138.9 U/l, range 5–1196 U/l). However, when considering only the 108 patients with evidence of disease and without medical treatment at study entry (ND 81 and RL patients 27), the IRMA assay did not

discriminate CgA levels of patients with metastases from those without (676.1 ± 1554.9 ng/ml, range 10–11, 270 ng/ml vs 272.9 ± 503.6 ng/ml, range 28–2715 ng/ml; $P=0.09$). On the other hand, CgA

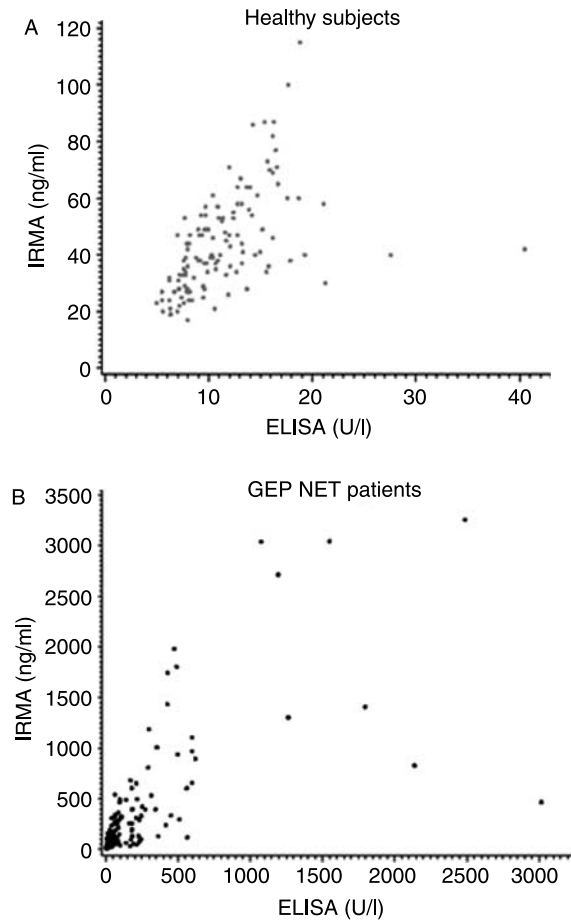


Figure 1 Comparison between ELISA and IRMA methods in 129 healthy subjects (A) and in 202 GEP NET patients (B).

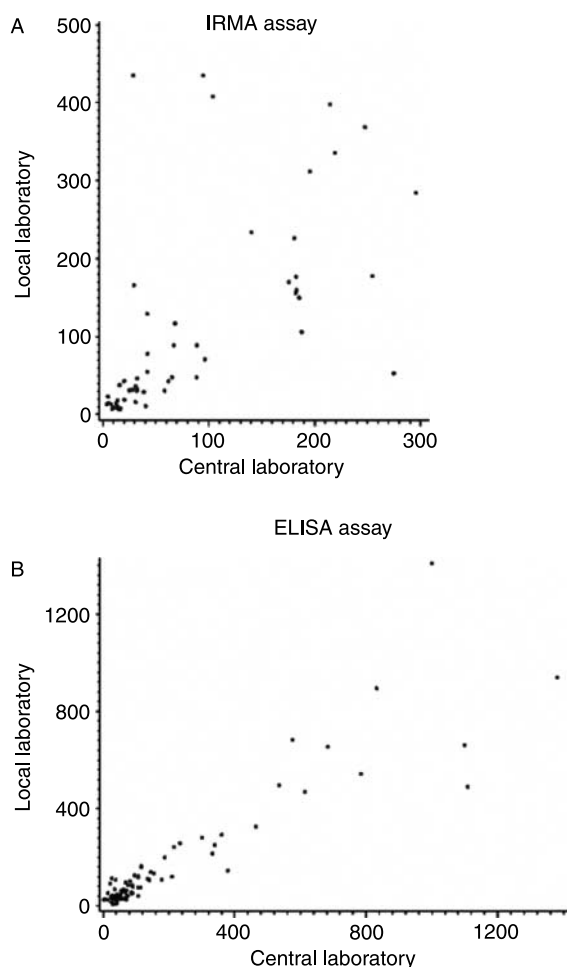


Figure 2 Comparison between local and central laboratories for IRMA (A) and ELISA (B) methods in GEP NET patients.

levels measured by the ELISA assay significantly differed in the two groups (450.4 ± 1073.2 U/l, range 5–8170 U/l vs 90.2 ± 222.4 U/l, range 6–1196 U/l; $P < 0.0002$).

In order to identify a CgA cutoff value that could distinguish between patients with metastases from those without, we performed a ROC analysis evaluating CgA levels from affected patients (ND+RL=108), excluding patients in RM and those with SD. Therefore, the ROC curve was constructed by considering CgA levels measured by both IRMA and ELISA assays in 79 vs 29 patients with and without metastases respectively.

As shown in Fig. 4A, with the IRMA assay, the cutoff value of 146 ng/ml provided the best compromise between specificity (55.6%) and sensitivity (57.0%), and was chosen for further analysis. The AUC was 0.613 indicating a modest performance of

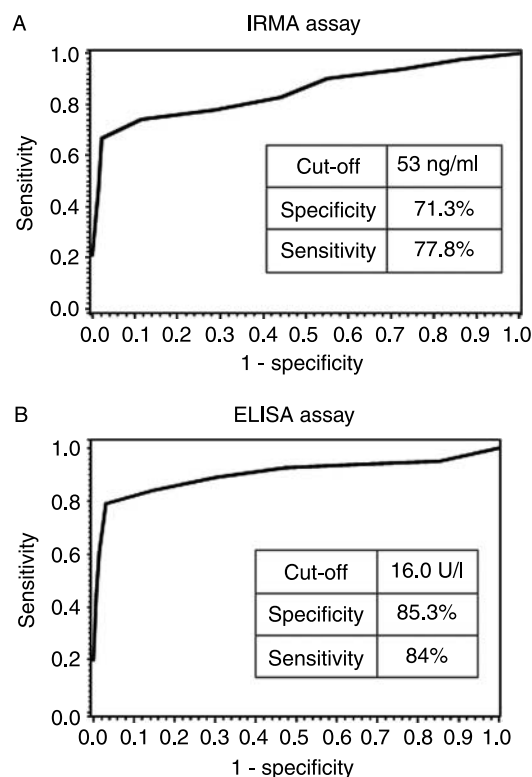


Figure 3 Receiver-operating characteristics curve obtained with 129 healthy subjects and 81 new diagnosis GEP NET patients for the IRMA (A) and for the ELISA assays (B).

the assay. Using this cutoff value, PPV were 49 and 40% and NPV were 72 and 46% for foregut and midgut tumors respectively. Analysis of these parameters was then performed according to the affected organ. Due to the low numerosity, PPV and NPV were only calculated for stomach (14 and 65%) and pancreas (84 and 78%) respectively.

As shown in Fig. 4B, with the ELISA assay, the cutoff value of 67.3 U/l provided the best compromise between specificity (71.4%) and sensitivity (63.3%), and was chosen for further analysis. The AUC was 0.727, indicating again a modest performance of the assay. Using this cutoff value, PPV were 61 and 58% and NPV were 76 and 65% for foregut and midgut tumors respectively. When considering the affected organ, it is worth to underline that for pancreatic NETs, with the chosen cutoff levels for both IRMA and ELISA assays, PPV was 84% and NPV was 78%, with 90% specificity and 68% sensitivity. On the other hand, PPV and NPV for stomach were 22 and 79% with the ELISA assay.

Table 3 shows CgA levels assessed both by IRMA and ELISA assays in 79 metastatic GEP NET patients, belonging to ND and RL groups, according to the

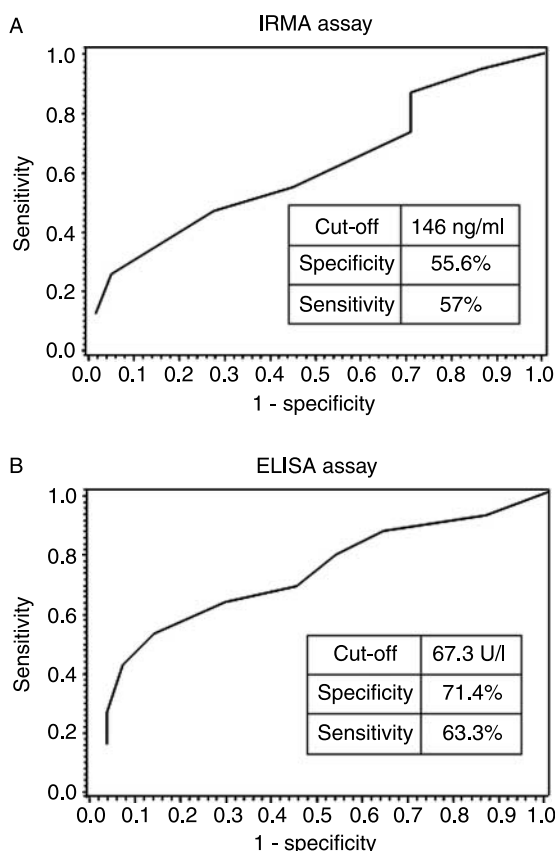


Figure 4 Receiver-operating characteristics curve obtained with 79 patients with metastases and 29 patients without metastases for the IRMA (A) and for the ELISA assays (B).

spread of the disease. Data on disease extension were missing for nine patients. CgA levels were greater in patients with liver metastases when compared with those with locally advanced disease. In addition, CgA levels, evaluated by both IRMA and ELISA assays, were lower in patients with extensive metastatic spread (extra-hepatic metastases; 194.8 ± 123.2 ng/ml, range 69–423 ng/ml; 81.5 ± 70.7 U/l, range 13–255 U/l) than in those with liver metastases only (800.9 ± 1206.7 ng/ml, range 36–4690 ng/ml; 515.2 ± 773.2 U/l, range 9–3018 U/l).

Discussion

In this multicenter observational study, we have focused on assessing the value of CgA as a biochemical marker for detection of GEP tumors. An important result of this study is that the employed assays for CgA measurement, which are the most commonly available in clinical practice, showed a very good correlation between them, in agreement with previous reports (Stridsberg *et al.* 2003), as well as a good correlation

between the values obtained by the central laboratories in Venezia and Orbassano and those obtained by the peripheral laboratories. Therefore, we can assume that CgA values reported by the laboratories of the 40 Italian centers participating in the study are as reliable as those obtained in dedicated laboratories and support the reliability of CgA evaluation in clinical practice.

The results show that CgA levels are highly variable in our study population, with overlapping levels between healthy subjects, patients with active disease (ND, RL, and SD) and patients in RM, as measured by both IRMA and ELISA assays, suggesting a modest diagnostic value for CgA assessment in the screening procedures for GEP NETs.

The limited diagnostic power of CgA measurement is also underlined by the results of the ROC analysis, performed by considering healthy subjects and ND patients. The analysis indeed identified cutoff values for IRMA and ELISA assays located between the 75th and the 95th percentile of the CgA values distribution in healthy controls, with modest sensitivity (77.8 and 84%) and specificity (71.3 and 85.3%) for both IRMA and ELISA assays respectively. This evidence is in line with previous reports showing a relatively low diagnostic value of circulating CgA in NETs (Nobels *et al.* 1998, Tomassetti *et al.* 2001). This may depend on type, secretory activity, degree of neuroendocrine differentiation, and total burden of the tumors (Seregini *et al.* 2001), as well as on the highly variable CgA levels of the control group. Indeed, 26 and 16% healthy subjects had high baseline CgA levels by IRMA and ELISA assays respectively, probably because of the many potential tissue sources of the peptide (Lamberts *et al.* 2001). Furthermore, chronic atrophic gastritis, which causes high-circulating CgA levels (Syversen *et al.* 2004), was not completely ruled out in our control group, even if all healthy subjects were asymptomatic.

The CgA cutoff levels identified by the ROC analysis in the present study are lower than those described in previous studies. Stridsberg *et al.* (2003) adopted the kit cutoff levels without validating them and considered a patients group including subjects who lacked signs of NET. Other authors calculated the cutoff levels on the basis of control groups lacking strict exclusion criteria (Ferrari *et al.* 2004, Nehar *et al.* 2004) or previously diagnosed with non-GEP NETs (Ferrari *et al.* 2004).

The PPV and NPV of CgA measurement for both IRMA and ELISA were calculated on the basis of the disease prevalence in our study group. Reliable epidemiological data concerning GEP NET, essential

Table 3 Basal chromogranin A levels in 79^a gastroenteropancreatic neuroendocrine tumor patients according to metastatic spread

	Locally advanced disease		Liver metastases		Liver and extra-hepatic metastases	
	IRMA (ng/ml)	ELISA (U/l)	IRMA (ng/ml)	ELISA (U/l)	IRMA (ng/ml)	ELISA (U/l)
No.	22	22	39	39	9	9
Mean	359.7	256.8	800.9	515.2	194.8	81.5
s.d.	412.8	449.4	1206.7	773.2	123.2	70.7
Median	199	65	327	219	146	60
Range	22–1410	5–1798	10–4990	8–3300	69–423	13–255
Percentile						
5th	27	5	36	9	69	13
25th	44	28	103	95	107	42
50th	199	65	327	219	146	60
75th	491	234	971	562	291	89
95th	1305	1266	4690	3018	423	255

^aData on disease extension is missing for nine patients.

to accurately identify PPV and NPV for CgA levels prevalence in Italy, are currently lacking. Therefore, correction for the disease prevalence in the general population was not performed. As a consequence, data analysis overestimates PPVs and underestimates NPVs, probably impairing the diagnostic value of these parameters. Our analysis shows quite low PPVs, therefore suggesting even lower PPVs when considering disease prevalence in the general population. Thus, the identified PPVs for CgA assessment cannot be considered reliable discriminators for disease presence. On the other hand, our analysis shows very high NPVs, therefore suggesting even higher NPVs when considering disease prevalence in the general population. Thus, identified NPVs for CgA assessment could be considered reliable discriminators for disease absence.

Twenty-eight out of 202 patients (13.9%) were classified differently by the two assays, suggesting that CgA assessment with only one out of the two assays is not sufficient to exclude the presence of increased CgA levels in these patients. However, the discordance rate observed in our database is much lower than that reported by Ferrari *et al.* (2004). We previously demonstrated that the discordance between the results of the two assays is not due to the use of different blood derivatives (Leon *et al.* 2005), but might be due to the different ability of the antibodies to detect CgA-derived peptides. Moreover, these findings support the hypothesis that the two CgA kits used may provide different information, since a 20.1% discordance rate was also found among healthy subjects.

In keeping with previous studies (Nehar *et al.* 2004), we found higher CgA levels in metastatic patients when compared with those without metastases.

However, our study does not demonstrate a statistically significant difference among patient groups with increasing metastatic spread as indicated by previous studies (Nobels *et al.* 1998, Peracchi *et al.* 2003). On the contrary, we found that CgA levels assayed with both methods were lower in patients with very extensive metastatic spread when compared with those having metastases limited to the liver. The influence of concomitant therapies can be excluded, since the analysis was performed on newly diagnosed patients, neither previously treated by surgery nor by medical therapy. Therefore, the lower CgA levels in patients with very extensive metastatic spread might be attributed to a possible loss of neuroendocrine differentiation, probably indicating a more aggressive behavior. It has been previously demonstrated that CgA is normally absent or only focally expressed in poorly differentiated endocrine carcinomas (Rindi & Klöppel 2004). However, the lack of complete information concerning proliferative index and histology in these tumors does not allow us to draw any definitive conclusion. Follow-up data are needed to better clarify this issue, also in the light of previous studies showing that elevated CgA levels are strongly correlated with tumor volume (Nobels *et al.* 1997) and disease extent (Seregini *et al.* 2001).

The ROC analysis identified a cutoff level of 146 ng/ml for the IRMA and of 67.3 U/l for the ELISA assays as discriminating between patients with metastases and those without, but sensitivity (57 and 63.3% respectively) and specificity (55.6 and 71.4% respectively) were quite low. On the other hand, the calculated NPVs of CgA measurement for both IRMA and ELISA assays are very high, suggesting that CgA

values below the identified threshold levels are highly indicative of the absence of metastases in foregut and midgut GEP NET patients. Correction for the prevalence of the disease in the general population would again result in higher NPVs, suggesting that the CgA cutoff levels of 146 ng/ml for IRMA and 67.3 U/l for ELISA could discriminate patients without metastases from metastatic patients. On the other hand, our analysis showed rather low PPVs for discriminating metastatic patients, indicating that values above the chosen cutoff levels are not predictive of the presence of metastases in the majority of cases, also because they are very likely overestimates. However, our data also point out that in patients with pancreatic tumors CgA levels, evaluated with both methods, have a high predictive value for the presence of metastases, since they correctly identify 84% of these patients as having metastatic disease, with good specificity (90%), but modest sensitivity (68%). These data suggest that in patients diagnosed with pancreatic NETs CgA evaluation might be useful to identify patients in which local and distant metastases should be looked for by an accurate clinical evaluation. This issue is important, since it has been previously demonstrated that in pancreatic NETs, the presence of metastases profoundly influences survival rate, which is significantly better in patients without metastases (Madeira *et al.* 1998, Chu *et al.* 2002, Gullo *et al.* 2003, Panzuto *et al.* 2005, Tomassetti *et al.* 2005). Moreover, the 5-year survival rate was reported to be 60–100% for localized disease, 40% for regional disease, 29% for distant metastases, and 80% for all stages (Eriksson *et al.* 1990, Modlin *et al.* 2003). Therefore, CgA evaluation could have a clinical value also for prognosis.

The study presented here also found for ELISA a higher sensitivity and specificity (84 and 85% respectively) when compared with IRMA assay (71.3 and 77.8% respectively) in identifying patients affected by NETs. The greater ELISA sensitivity might be due to a more extensive CgA cleavage by GEP NETs. Indeed, the IRMA assay mainly evaluates intact molecules and major CgA fragments, since it employs two antibodies recognizing the central part of human CgA, which is unexposed to proteolysis (Degorce *et al.* 1999, Bernini *et al.* 2001). In pathological conditions, such as NETs, different proteolytic processes may take place, generating a variable number of fragments (Taupenot *et al.* 2003), which are better assessed by the ELISA assay. However, further studies are needed to address the specific CgA cleavage in different tumors. Initial proteomic studies have identified 11 novel CgA-derived peptides in endocrine tumors,

supporting the hypothesis that different tumors may process differently the entire molecule, representing a possible specific signature (Orr *et al.* 2002).

In conclusion, our study shows that an accurate comparison between healthy subjects and GEP NET patients does not provide cutoff levels that could discriminate between the two groups with a sensitivity and a specificity high enough to demonstrate CgA as an efficient biochemical marker in the diagnostic screening of GEP NET. These data indicate that CgA serum levels can be helpful for the clinical management of NETs, but with low sensitivity and specificity for diagnostic purposes. On the other hand, the main utility of CgA measurement may be in patient monitoring. Therefore, follow-up prospective data are necessary to examine the performance of CgA assessment in evaluating follow-up and treatment efficacy in GEP NET patients. Further studies are ongoing to clarify this issue.

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